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Cotransplantation of Mesenchymal Stem Cells Might Prevent Death from Graft-versus-Host Disease (GVHD) without Abrogating Graft-versus-Tumor Effects after HLA-Mismatched Allogeneic Transplantation following Nonmyeloablative Conditioning

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Recent studies have suggested that coinfusion of mesenchymal stem cells (MSCs) the day of hematopoietic cell transplantation (HCT) might promote engraftment and prevent graft-versus-host disease (GVHD) after myeloablative allogeneic HCT. This prompted us to investigate in a pilot study whether MSC infusion before HCT could allow nonmyeloablative (NMA) HCT (a transplant strategy based nearly exclusively on graft-versus-tumor effects for tumor eradication) from HLA-mismatched donors to be performed safely. Twenty patients with hematologic malignancies were given MSCs from third party unrelated donors 30-120 minutes before peripheral blood stem cells (PBSCs) from HLA-mismatched unrelated donors, after conditioning with 2 Gy total body irradiation (TBI) and fludarabine. The primary endpoint was safety, defined as a 100-day incidence of nonrelapse mortality (NRM) <35%. One patient had primary graft rejection, whereas the remaining 19 patients had sustained engraftment. The 100-day cumulative incidence of grade II-IV acute GVHD (aGVHD) was 35%, whereas 65% of the patients experienced moderate/severe chronic GVHD (cGVHD). One-year NRM (10%), relapse (30%), overall survival (OS) (80%) and progression-free survival (PFS) (60%), and 1-year incidence of death from GVHD or infection with GVHD (10%) were encouraging. These figures compare favorably with those observed in a historic group of 16 patients given HLA-mismatched PBSCs (but no MSCs) after NMA conditioning, which had a 1-year incidence of NRM of 37% ($P = .02$), a 1-year incidence of relapse of 25% (NS), a 1-year OS and PFS of 44% ($P = .02$), and 38% ($P = .1$), respectively, and a 1-year rate of death from GVHD or infection with GVHD of 31% ($P = .04$). In conclusion, our data suggest that HLA-mismatched NMA HCT with MSC coinfusion appeared to be safe.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) following nonmyeloablative (NMA) conditioning has been an effective treatment for many patients with hematologic malignancies who have an HLA-matched related or unrelated donor [1-10]. However, results of NMA HCT in patients with HLA-mismatched donors have been less favorable because of a high incidence of graft rejection and severe acute graft-versus-host disease (aGVHD) [4,5,11-13]. NMA HCT relies nearly exclusively on graft-versus-tumor (GVT) effects for tumor eradication, which has been closely correlated with both achievement of full donor T cell and natural killer (NK) cell chimerism

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The first 2 authors contributed equally to this study.

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[14-17] and occurrence of chronic GVHD (cGVHD) [14,18-21].

Mesenchymal stem cells (MSCs) are multipotent progenitors within the bone marrow (BM) capable of differentiating into various cells and tissues, such as chondrocytes, osteoblasts, and adipocytes [22]. In vitro, MSCs support hematopoiesis, and inhibit T cell proliferation [23], NK cell cytotoxicity [24], and dendritic cell differentiation [25,26]. In animal models, coinfusion of MSCs has been shown to facilitate engraftment of human cord blood $CD34^+$ cells in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice [27], to prolong skin allograft survival in baboons [28], and to prevent aGVHD in some mice models [29] as well as in a xenogeneic (human into NOD/SCID mice) models of aGVHD [30], perhaps by promoting the generation of regulatory T cells [29,31]. In addition, phase II studies in humans have demonstrated that MSC infusions were safe [32-34], and might accelerate lymphocyte recovery and prevent graft failure after haploidentical HCT [35]. Further, MSC infusions have also shown promising efficacy in patients with steroid-refractory aGVHD [36], although a recent industry-sponsored trial failed to shown improvement of survival by MSCs in that setting [37]. These observations prompted us to investigate whether MSC infusion 0.5 to 2 hours before HCT could allow NMA HCT from HLA-mismatched donors to be performed safely (defined as a 100-day incidence of nonrelapse mortality [NRM] <35%). Because MSCs coinfusion has been shown to promote tumor growth in a mice study [38], and has been associated with a significantly increased relapse rate in 1 human study [39], an important additional focus of our study was to examine the impact of MSC coinfusion on cGVHD, and more importantly on GVT effects.

PATIENTS AND METHODS

Eligibility Criteria

Patients

Patients older than 55 years of age, and those with comorbid conditions precluding myeloablative conditioning were eligible for the study if they had 1 of the following hematologic malignancies: acute leukemia in complete remission, chronic myelogenous leukemia unresponsive to imatinib (or imatinib intolerance) but not in blast crisis, myeloproliferative disorder not in blast crisis and not with extensive myelofibrosis, myelodysplastic syndrome with <5% BM blasts at the time of HCT, high risk multiple myeloma (MM), chronic lymphocytic leukemia, or lymphoma. Exclusion criteria included HIV seropositivity, age of >75 years, terminal organ failure except for renal failure (dialysis acceptable), uncontrolled infection, arrhythmia or hypertension, previous radiation therapy precluding the use of 2 Gy total body irradiation (TBI), and presence of a HLA-matched donor fit to donate PBSC.

PBSC donors

Related or unrelated donors who had 1-2 HLA mismatches, as either: 1 antigenic mismatch at HLA-A or -B or -C or -DRB1 or -DQB1; 1 allelic mismatch at HLA-A or -B or -C or -DRB1 or -DQB1; 2 allelic mismatches at HLA-A or -B or -C or -DRB1 or -DQB1; 1 antigenic mismatch $\pm$ 1 allelic mismatch at HLA-A or -B or -C or -DRB1 or -DQB1; 1 antigenic mismatch at -DQB1 and 1 other antigenic mismatch at HLA-A or -B or -C or -DRB1 could serve as peripheral blood stem cell (PBSC) donor. Compatibility between donor and recipient for HLA-A, -B, -C, -DR, and -DQ was assessed by high-resolution techniques. Only PBSCs were allowed as stem cell source. Unrelated PBSCs were collected according to the procedures of the respective National Donor Registries.

MSC donors

Inclusion criteria for MSC donors included: related (sibling, parent, or child) or unrelated to the recipient (all MSC donor in the current study were actually unrelated to the recipients); aged >16 years; no HLA matching required; fulfilled generally accepted criteria for allogeneic HSC donation; and informed consent given. Exclusion criteria included: HIV seropositivity, known allergy to lidocaine, and any risk factor for transmissible infectious diseases. Compatibility between MSC donor, PBSC donor, and recipient for HLA-A, -B, -C, -DR, and -DQ was assessed by low-resolution techniques.

The protocol was approved by the local Ethics Committee and by the Belgian Federal Agency for Medicines and Health Products (Eudract # 2006-004101-26). Written informed consent was obtained for each patient and MSC donor. The study was also registered to ClinicalTrials.gov (protocol # NCT00504803).

MSC Preparation

MSC expansion cultures were performed at the University of Liège as described by other groups of investigators [36]. Briefly, BM (30-50 mL) was collected under local anesthesia in sterile conditions, and put in sterile heparin-containing syringes. Mononuclear BM cells were isolated by Ficoll (GE Healthcare-Amersham Biosciences AB, Upsala, Sweden), seeded in sterile tissue culture flasks (BD Falcon, Bedford, MA), and cultured in Dulbeccós Modified Eagles Medium-Low Glucose (Invitrogen, Merelbeke, Belgium) with glutamate supplemented

with 10% irradiated fetal bovine serum (Hyclone-Perbio Science, Merelbeke, Belgium) and antibiotics (penicillin/streptomycin, Lonza Bio Science, Verviers, Belgique). Cultures were maintained at 37 °C in humidified atmosphere containing 5% CO_2 for a total of about 4 weeks. The medium was replaced twice a week and, after approximately 2 weeks, the cultures were near confluence (>70%). Cells were then detached by treatment with irradiated trypsin-EDTA (Invitrogen, Merelbeke, Belgium) and replated (passaged) at a lower density to allow further expansion. A second passage was performed when the cells reached again confluence (>70%). After a sufficient 2 passages, the cells were harvested, washed, and resuspended using phosphate-buffered saline (PBS)-EDTA (Miltenyi Biotec, Utrecht, The Netherlands) and Human Serum Albumin (HSA) (CDF-CAF, Bruxelles, Belgique). MSCs were then frozen in a medium containing 70% PBS, 20% HSA, and 10% DMSO (WAK-Chemie, Steinbach, Germany) by standard techniques. Before infusion, MSCs were thawed and diluted in PBS, and then injected into the patients 30-120 minutes before PBSCs. All reagents were certified sterile, and endotoxin-free, and had been used in other clinical trials in Europe. In addition, the batch of fetal bovine serum used was selected after extensive testing, and was irradiated to ensure removal of all potential viruses. The following analyses were performed as quality controls for each MSC expansion culture: nucleated cell count on a manual cell counter, flow cytometry analysis with determination of the % cells (on total cells) positive for CD73, CD90, and CD105, and negative for HLA-DR, CD31, CD80, CD14, CD45, CD3, and CD34; cell viability by trypan blue exclusion; microbiology testing, including standard virology, bacterial culture, and search for mycoplasma; endotoxin detection by the limulus test; and cytogenetics. Further, MSC differentiation into adipocytes, osteocytes, and chondrocytes as well as inhibitory effects of MSCs on T cell function were validated in preliminary experiments, and verified after some MSC expansion. In the current study, MSC donors were third-party unrelated to the recipient and to the PBSC donor in all cases.

Conditioning Regimen, HCT, and Post-HCT Follow-up

The conditioning regimen consisted of Flu 30 mg/m^2 on days −4, −3, and −2 (total dose 90 mg/m^2), followed by a single dose of 2 Gy TBI administered on day 0 before infusion of cells. MSCs were infused first, followed by PBSCs infused at least 30-120 minutes later. Mycophenolate mofetil (MMF) was administered orally from the evening of day 0 through day 42 at the dose of 15 mg/kg three times a day. Tacrolimus was given orally at the dose of 0.06 mg/kg twice a day starting on day –3. The dose was then adapted according to through whole blood values between 15 and 20 ng/mL the first 28 days, and between 10 and 15 thereafter. Full doses were given until day 180. Doses were then progressively tapered to be definitely discontinued by day 365 in the absence of GVHD. Tacrolimus was discontinued earlier in case of disease progression or graft rejection, in patients without disease response on day 100, and in those with very-high risk disease. Tacrolimus was continued longer in case of cGVHD.

The diagnosis, clinical grading, and treatment of aGVHD were performed according to established criteria for NMA HCT [14,40]. Diagnosis and grading of cGVHD were performed using the National Institutes of Health (NIH) consensus criteria [41]. Treatment was given for grade II-IV aGVHD and for extensive cGVHD. Initial treatment usually consisted of prednisolone, 1-2 mg/kg/day, with taper initiated within 14 days. In addition, tacrolimus was usually resumed at full doses. Steroid-refractory aGVHD was treated as per available investigational protocols or standard practice. Treatment of cGVHD consisted of methylprednisolone (1 mg/kg) with alternate-day tacrolimus. Steroid-refractory cGVHD was generally treated with rapamycine, MMF, or photophoresis.

Standard prophylaxis against infections was used [42]. Disease evaluation was routinely carried out on days 40, 100, 180, 365, and then at least yearly thereafter. Persistent, progressive or relapsed malignancies in the absence of severe manifestation of GVHD were treated by rapid taper and discontinuation of immunosuppression to initiate GVT effects, and with chemotherapy with or without added antimyeloma agents such as thalidomide, lenolidomide, or bortezomib according to the underlying disease.

Chimerism Analyses

Chimerism among T cells and total white blood or BM cells was assessed on days 28, 42, 100, 180, and 365 after HCT using PCR-based analysis of polymorphic microsatellite regions (AmpFlSTR® Identifiler®, Applied Biosystems, Lennik, Belgium). CD3 (T cells) selection was carried out with the RosetteSep lymphoid enrichment kit (StemCell Technologies, Grenoble, France). Graft rejection was defined as the occurrence of <5% T cells of donor origin after HCT, as previously described [3,15]. Numbers of T cells of donor origin were calculated by multiplying the absolute numbers of $CD3^+$ T cells by the % of T cells of donor origin (chimerism) on the same day.

Historic Group

Sixteen consecutive patients given HLA-mismatched unmanipulated PBSCs from unrelated donors following Flu and 2 Gy TBI between May 2002 and August 2006 were included in a historic

control group (we thus excluded patients given HLA-mismatched CD8-depleted PBSCs [5] and those conditioned with 4 Gy TBI, to have a historic group as comparable as possible to the study group). Their diagnosis included MM (n = 6), follicular lymphoma (n = 3), mantle cell lymphoma (n = 2), acute myelogenous leukemia (AML) in complete remission (n = 2), myelodysplastic syndrome (refractory anemia n = 1, refractory anemia with excess of blasts n = 1), and renal cell carcinoma (n = 1) (Table 1). Median patient age was 55 years (range: 10-69 years). Eleven pairs were mismatched for at least 1 HLA antigen (including 3 pairs who were also mismatched for another HLA antigen [n = 1], or another HLA allele [n = 2]), whereas 5 pairs were mismatched for a single HLA class I (n = 2) or HLA class II (n = 3) alleles. Median HCT-comorbidity index (HCT-CI) score [43] was 3 (range: 0-6). Median number of transplanted $CD34^+$ and $CD3^+$ T cells were 4.1 (range: 1.5-20.2) $\times$ 10^6/kg recipient and 313 (range: 159-790) $\times$ 10^6/kg recipient, respectively. Postgrafting immunosuppression and infection prophylaxis was similar to what was done in the MSC group. Specifically, postgrafting immunosuppression included MMF administered orally from the evening of day 0 through day 42 at the dose of 15 mg/kg three times a day. Tacrolimus (n = 5) or cyclosporine (n = 11) were given orally at full doses until day 180, and then progressively tapered to be definitely discontinued by day 365.

Statistical Analyses

Results were analyzed as of October 23, 2009. Survival and progression-free survival were estimated by the Kaplan-Meier method. Cumulative incidence estimates for GVHD, relapse, NRM, death from GVHD or infection while on treatment for GVHD, and graft rejection were calculated using methods previously described [44]. The primary endpoint was to study the feasibility and safety (defined as a day 100 incidence of NRM $\leq$35%) of NMA HCT with coinfusion of MSCs and HLA-mismatched PBSCs. There was a stopping rule for evidence for NRM >35%. The impact of MSC cotransplantation on survival, relapse, and treatment-related mortality was assessed in multivariate Cox models adjusted for type of HLA mismatch (1 single allele versus >1 single allele), comorbidity [43], disease risk [45], and patient age. Statistical analyses were

Table 1. Characteristics of Patients and HCT Outcomes

	MSC Group	Historic Group	*P* Value
Number of patients	20	16	NS
Age; median (range), years	58 (21-69)	55 (10-69)	NS
Gender (male/female); No. of patients	14 / 6	13 / 3	NS
Disease at transplantation; No. of patients			
Acute myelogenous leukemia	7	2	
Chronic lymphocytic leukemia	1	0	
Non-Hodgkin lymphoma	5	5	
Hodgkin lymphoma	1	0	
Myelodysplastic syndrome	0	2	
Multiple myeloma	5	6	
Plasmablastic leukaemia	1	0	
Metastatic renal cell carcinoma	0	1	
Disease risk*: low / standard / high ; No. of patients	6 / 11 / 3	7 / 4 / 5	NS
Patient/donor compatibility; No. of patients			NS
≥1 HLA-antigen mismatch	13	11	
2 HLA-allele mismatches	1	0	
1 HLA-allele mismatches	6	5	
Comorbidities (HCT-CI score†); median (ranges)	3 (0-9)	3 (0-6)	NS
Karnofsky score; median (ranges)	90 (50-90)	90 (70-90)	NS
Prior autologous HCT, No. of patients	10	9	NS
No. of cells transplanted ($\times 10^6$/kg); median (range)			
$CD34^+$ cells	4.8 (1.6-11.8)	4.1 (1.5-20.2)	NS
$CD3^+$ cells	312 (120-540)	313 (159-790)	NS
Graft rejection‡; No. of patients	1	0	NS
Number of T cells of donor origin on day 28; median (range)	293 (4-540)	202 (41-886)	NS
Incidence of grade II-IV acute GVHD (%)	45	56	NS
Grade IV acute GVHD, # of patients (%)	2 (10)	3 (19)	NS
1-year probability of dying from GVHD or infection while on treatment for GVHD (%)	10	31	.04
1-year nonrelapse mortality (%)	10	37	.02
1-year relapse incidence (%)	30	25	NS
1-year progression-free survival (%)	60	38	.1
1-year overall survival (%)	80	44	.02

GVHD indicates graft-versus-host disease; HCT, hematopoietic cell transplantation; msc, mesenchymal stem cell; HCT-CI, HCT-comorbidity index.
*-reference #45.
†-reference #43.
‡-occurrence of <5% T cell of donor origin after HCT.

Table 2. Detailed Patients, Donors and HCT Characteristics in the MSC Group (All Patients Were Given Grafts from Unrelated Donors)

Pt No.	Pt Age (Years)/Gender	Disease/Status at HCT	Indication for Allogeneic HCT	HCT-CI Score /KS	Donor Age (Years)/ Gender	M/M in Rejection Direction	M/M in GVHD Direction	No. CD34 Transplanted ($\times 10^6$/ kg)	No. CD3 Transplanted ($\times 10^6$/ kg)
1	58 / F	MM / PR	7 prior lines of θ including 2 auto-HCT	1/ 70	41 / M	0	DQB1 (1Al)	11.8	312
2	41 / F	CLL / PR	Del 17p, refractory to fludarabine-cyclophosphamide	3 / 100	50 / M	C (1Ag)	C (1 Ag)	4.3	266
3	65 / F	AML / CR1	FLT3-ITD	0 / 100	44 / F	C (1Ag)	C (1 Ag)	2.1	350
4	58 / M	MM / VGPR	2 auto-HCT	3 / 100	37 / M	C (1Ag)	C (1 Ag)	8.1	222
5	65 / M	PL / CR1	2 auto-HCT	3 / 80	29 / M	C (1Ag)	C (1 Al)	3.1	164
6	43 / M	NHL-T / CR1	1 auto-HCT	3 / 90	19 / M	C (1Ag)	0	1.6	121
7	61 / M	AML / CR1	Secondary to extended field radiotherapy for seminoma	4 / 90	36 / F	DRB1 (1 Al)	DRB1 (1 Al)	7.1	364
8	21 / M	AML / CR2	ARDS, prior HCT, invasive lung aspergilosis at HCT	4 / 80	36 / M	DQB1 (1 Al)	DRB1 (1 Al)	6.3	540
9	61 / M	AML / CR2	Del 7q, auto-HCT	2 / 90	43 / M	C (1Ag)	C (1 Ag), DQB1 (1Ag)	6.4	390
10	56 / F	AML / CR1	Secondary to chemoradiotherapy for breast cancer	6 / 50	42 / M	C (1Ag)	C (1 Ag) DRB1 (1 Al) DQB1 (1 Al)	4.7	297
11	64 / M	NHL-F / ref.	5 prior lines of θ including auto-HCT	0 / 90	50 / F	C (1Ag)	C (1 Ag)	7.8	429
12	55 / M	AML / CR1	Secondary to chemo-radiotherapy for sarcoma	9 / 80	22 / M	DQB1 (1 Al)	DQB1 (1 Al)	2.6	275
13	69 / M	MCL / CR1	Failure of autologous PBSC mobilisation	1 / 90	41 / M	B (1 Al)	B (1 Al)	5.1	354
14	60 / M	DLBCL / CR2	Failure of autologous PBSC mobilisation	3 / 80	43 / M	C (1Ag)	C (1 Ag)	3.8	408
15	30 / M	HD / CR1	CR obtained only after auto-HCT	3 / 90	28 / M	B (1 Al)	B (1 Al)	3.6	120
16	49 / M	MM / PR	4 prior lines of θ including 2 auto-HCT	2 / 90	37 / M	0	C (1 Ag)	8.9	303
17	56 / F	AML / CR1	Normal cytogenetics with NPM1 unmutated	0 / 90	35 / M	C (1 Ag)	C (1 Al)	9.2	324
18	64 / F	MM / VGPR	6 prior lines of θ including 3 auto-HCT	3 / 80	25 / M	C (1Ag)	C (1 Ag)	9.1	267
19	54 / M	DLBCL / CR	CR obtained only after auto-HCT	2 / 100	49 / F	B (1 Al)	B (1 Al)	4.8	314
20	62 / M	MM / VGPR	4 prior lines of θ including 2 auto-HCT	3 / 90	32 / M	C (1 Ag), B (1 Al)	C (1 Ag), B (1 Al)	3.1	133

Pt indicates patient; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation comorbidity index; KS, Karnofsky score; M/M, mismatch; GVHD, graft-versus-host disease; F, female; M, male; MM, multiple myeloma; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; PL, plasmoblastic leukemia; NHL-T, T cell non-Hodgkin lymphoma; NHL-F, follicular non-Hodgkin lymphoma; sAML, secondary AML; PBSC, peripheral blood stem cell; DLBCL, Diffuse large B cell lymphoma; HD, Hodgkin Disease; PR, partial response; CR, complete remission; VGPR, very good partial response; ref, refractory; Ag, antigen; Al, allele; θ, treatment.

carried out with Graphpad Prism (Graphpad Software, San Diego, CA) and SAS version 9.1 for Windows (SAS Institute, Cary, NC).

RESULTS

Patients and PBSC Donors

Twenty patients were included in the study between January 2007 and September 2008. Their diagnoses are listed in Tables 1 and 2. Median patient age was 58 years (range: 21-69 years), and median HCT-CI score [43] was 3 (range: 0-9). Contraindication for myeloablative allogeneic HCT included aged ≥55 years (n = 6), previous autologous HCT (n = 4), aged ≥55 years and previous autologous HCT (n = 8), and comorbidity (severe mental deficiency [n = 1], and invasive lung aspergillosis [n = 1]). All patients were given grafts from unrelated donors. HLA-compatibility between donor and recipients is described in Table 1. Briefly, 13 pairs were mismatched for at least 1 HLA antigen (including 4 pairs who were also mismatched for another antigen [n = 3] or 1 HLA allele [n = 1]), 1 pair was mismatched for 2 HLA alleles, and 6 pairs were mismatched for a single HLA class I (n = 3) or HLA class II (n = 3) alleles. Median number of transplanted $CD34^+$ and $CD3^+$ T cells were 4.8 (range: 1.6-11.8) $\times$ 10^6/kg recipients and 312 (range: 120-540) $\times$ 10^6/kg recipients, respectively. Median follow-up for surviving patients was 560 (range: 398-908) days.

Mesenchymal Stem Cells

Before freezing, MSC viability was 93% (range: 90%-97%), whereas CD3 and CD45 were expressed by <0.01% of the cells, CD80 and CD31 by ≤1% of the cells, and CD90, CD105, and CD73 by 99% (range: 99%-100%), 99% (range: 98%-99%), and 99% (range: 88%-100%) of the cells, respectively. HLA compatibility between MSC donors, recipients, and PBSC donors was assessed for 17 of 20 patients. Number of HLA-antigen mismatches between MSC donors and recipients were 10 for 1 patient, 9 for 4 patients, 8 for 6 patients, 7 for 3 patients, 6 for 2 patients, and 3 for 1 patient. The number of HLA-antigen mismatches between MSC donors and PBSC donors were 10 for 1 patient, 9 for 4 patients, 8 for 5 patients, 7 for 4 patients, 6 for 2 patients, and 3 for 1 patient.

NRM (Primary Endpoint)

Two of 20 patients died of nonrelapse causes on days 74 (cerebral toxoplasmosis in a patient on steroids as treatment for grade II aGVHD) and 114 (encephalopathy and grade IV aGVHD) after HCT. Day 100 (primary endpoint) and day 365 cumulative incidence of NRM were 5% and 10%, respectively.

Engraftment Kinetics and Graft Rejection

One patient (patient #10, given 10/10 HLA-antigen mismatched MSCs) had primary graft rejection together with relapse of AML. All remaining patients had sustained donor T cell engraftment. Median donor T cell chimerism levels on days 28, 40, 100, 180, and 365 were 90%, 91%, 98%, 97%, and 98%, respectively (Figure 1). Interestingly, 1 patient experienced graft failure despite persistent high donor T cell (98.5%) and total white blood cell (99.7%) chimerism levels, and no evidence of disease relapse (but aplastic marrow) on day 267 after HCT. The patient is currently doing very well in complete remission without immunosuppressive treatment after receiving a second transplant from another HLA-mismatched donor after conditioning with Flu (90 mg/m^2) and 4 Gy TBI [46] on day 323 after his first transplant.

Acute GVHD and cGVHD

The 100-day incidence of grade II-IV aGVHD was 35%, but 2 patients had late aGVHD (after reinduction chemotherapy [n = 1], or donor leukocyte infusion (DLI) [n = 1]) (Figure 2A). Specifically, grades I, II, III, and IV aGVHD were seen in 2, 5, 2, and 2 (including the patient who developed GVHD following DLI) patients, respectively, whereas 2, 5, and 8 patients experienced NIH mild, moderate, and severe cGVHD, respectively. The 1-year cumulative incidence of moderate or severe cGVHD was 65% (Figure 2B). The 1- and 2-year probability of dying from GVHD or from infection while on treatment for GVHD were 10% and 10%, respectively.

Disease Response and Disease Progression

Three of 7 (43%) patients with measurable disease at HCT achieved complete remission on days 41, 104, and 353 after HCT, respectively. Two patients with AML (including the 1 with primary graft rejection) and 5 with MM experienced disease progression. The 1-year cumulative incidence of disease progression was 30%.

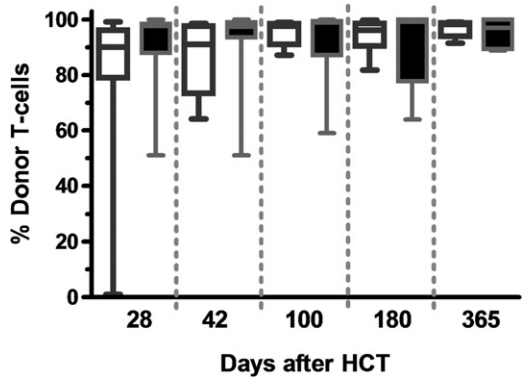

Figure 1. T cell engraftment kinetics (donor T cell chimerism levels) in MSC patients (white bars) and in historic patients (black bars).

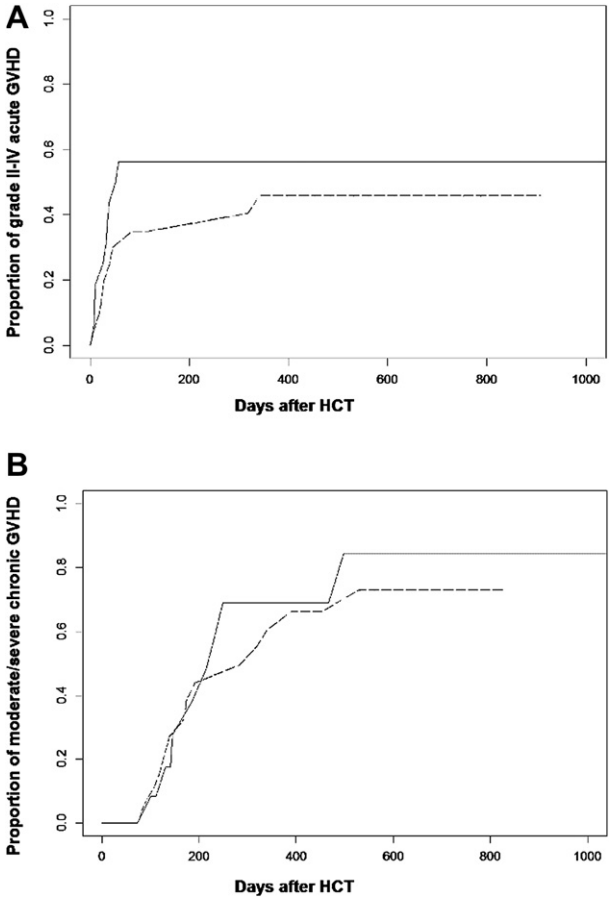

Figure 2. (A) Cumulative incidence of grade II-IV aGVHD in study patients given MSC (MSC group, broken line) or in historic patients (solid line). (B) Cumulative incidence of moderate/severe cGVHD in study patients given MSC (MSC group, broken line) or in historic patients (solid line).

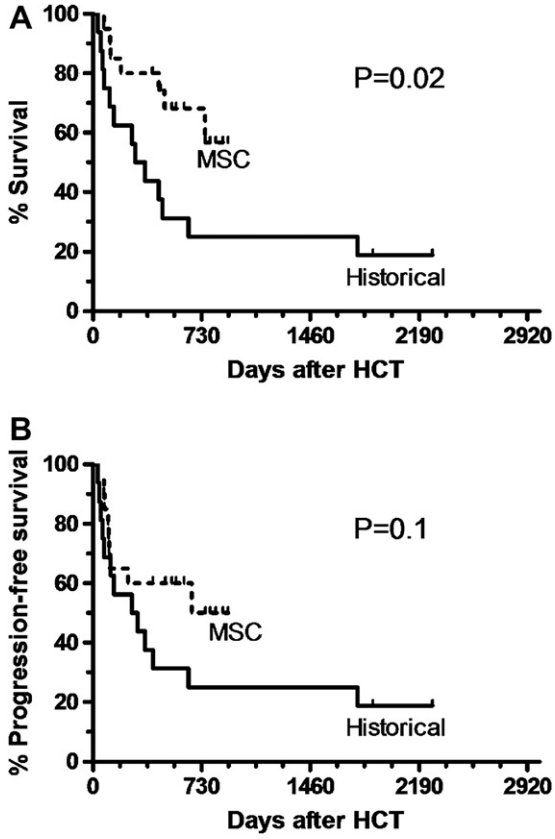

Figure 3. OS (A) and PFS (B) in study patients given MSC (MSC group, broken line) or in historic patients (solid line).

Overall Survival (OS) and Progression-Free Survival (PFS)

One-year OS and PFS were 80% and 60%, respectively (Figure 3A-B).

Comparison with the Historic Group

One of 20 patients in the MSC group versus 0 of 16 patients in the historic group experienced graft rejection. Median times to achieve 1×10^9 neutrophils/L and 100×10^9 platelets/L were 10 and 11 days in the MSC group, versus 9 ($P = .2$) and 13 ($P = .7$) days in the historic group, respectively. Median numbers of T cells of donor origin on day 28 posttransplant were 293 (range: 4-540) $\times 10^6$/L in the MSC group versus 202 (range: 41-886) $\times 10^6$/L in the historic group. Nine of 20 patients (45%) in the MSC group versus 9 of 16 patients (56%) in the historic group experienced grade II-IV aGVHD (Figure 2A). Two of 20 patients (10%) in the MSC group (including 1 patient after DLI) versus 3 of 16 patients (19%) in the historic group developed grade IV aGVHD. The 1- and 2-year probability of dying from GVHD or infection while on treatment for GVHD was 10% and 10%, respectively, in the MSC group, versus 31% and 38%, respectively, in the historic group ($P = .04$). One-year nonrelapse mortality, relapse incidence, OS, and PFS were 10%, 30%, 80%, and 60%, respectively, in the MSC group, versus 37% ($P = .02$), 25% ($P = .9$), 44% ($P = .02$), and 38% ($P = .1$) in the historic group (Figure 3A-B). Within the historic group, 1-year survival and incidence of death from GVHD or infection with GVHD were 45% and 27%, respectively, in patients given cyclosporine plus MMF as GVHD prophylaxis (n = 11), versus 40% and 40% in those given tacrolimus plus MMF as GVHD prophylaxis (n = 5). In multivariate analysis, MSC cotransplantation was significantly associated with decreased NRM [HR = 0.2 (95% confidence interval [CI], 0.04-0.9), $P = .03$] and overall mortality [HR = 0.4 (95% CI, 0.1-0.9), $P = .03$] (Table 3).

DISCUSSION

Previous studies have shown that MSC coinfusion might support engraftment of donor hematopoiesis/lymphopoiesis [35], and prevent severe aGVHD [39]. However, observations in mice and in humans have suggested that MSCs might abrogate graft-

Table 3. Multivariate Analysis of Factors Affecting Overall Mortality and Nonrelapse Mortality in the MSC and Historic Groups Combined (n = 36)

	Nonrelapse mortality		Mortality	
Factor	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Mesenchymal stem cell infusion	0.18 (0.04-0.87)	.03	0.35 (0.14-0.90)	.03
Patient age	1.0 (0.95-1.0)	.9	1.0 (0.99-1.1)	.3
Disease-risk (Kahl score)	0.6 (0.27-1.5)	.3	1.0 (0.6-1.8)	.9
Comorbidity (HCT-CI score)	1.0 (0.69-1.3)	.8	1.0 (0.8-1.3)	.8
>1 single allele HLA-mismatch	0.7 (0.16-2.8)	.6	0.9 (0.4-2.4)	.9

CI indicates confidence interval; MSC, mesenchymal stem cell; HCT-CI, hematopoietic cell transplantation comorbidity index.

versus-host alloreactivity, and promote disease relapse [38,39]. Because following NMA conditioning tumor eradication depends nearly exclusively on GVT effects [3,14,19], our study offers a unique opportunity to directly assess the impact of MSC cotransplantation on GVHD and GVT effects.

The primary endpoint of the study was to investigate whether NMA conditioning followed by cotransplantation of MSCs and HLA-mismatched PBSCs was safe (defined by a day 100 incidence of nonrelapse mortality <35%). This objective was largely achieved as the incidence of nonrelapse mortality was 5% at day 100, and 10% at 1 year. For comparison, the 1-year nonrelapse mortality was 37% in our historic group of patients given HLA-mismatched PBSCs, and ranged from 18% to 29% in other studies analyzing data from patients given HLA-matched unrelated PBSCs (without MSCs) after Flu and 2 Gy TBI [1,4,47,48]. The decreased NRM in the MSC group compared to the historic group is not likely to be related to the larger use of tacrolimus in the MSC group than in the historic group, because death from GVHD or infection was slightly higher in historic patients given tacrolimus plus MMF than in those given cyclosporine + MMF as GVHD prophylaxis.

Another important observation was that the rate of grade IV aGVHD was low in our study, and comparable to what has been observed with patients given HLA-matched unrelated PBSCs (without MSCs) after Flu and 2 Gy TBI [1,4,47,48]. Further, the rate of grade IV aGVHD as well as the incidence of death from or with GVHD was lower in our MSC group than in our historic group. These observations suggest that MSCs might have prevented death from GVHD in patients given HLA-mismatched PBSCs from unrelated donors.

A major observation of our study was that MSC coinfusion did not abrogate GVT effects. Following NMA conditioning, previous studies have demonstrated a close relationship between GVT effects and achievement of full donor T cell/NK cell chimerism [14,15,17], or occurrence of cGVHD [14,19]. In the current study, full donor T cell chimerism was achieved promptly in most patients, whereas 65% of patients experienced NIH moderate/severe cGVHD. Consequently, 43% of patients with measurable disease achieved a complete remission 41 to 353 days after HCT (a rate similar to what has been observed in patients given HLA-matched PBSCs without MSC coinfusion [14]), whereas the 1-year cumulative incidence of relapse was 30%, which is similar to what was observed in our control group.

Finally, we observed a 5% incidence of graft rejection (0% in the historic group), whereas donor T cell chimerism levels were similar in the MSC and historic groups. Further studies are needed to analyze the impact of MSC cotransplantation on chimerism levels and on the incidence of graft rejection after NMA conditioning, because one could speculate that MSCs could interfere with graft-versus-host reactions required to create BM space allowing establishment of donor hematopoiesis [49]. On the other hand, MSC might mitigate host-versus-graft rejection mediated by recipient T cells after NMA conditioning [49,50], thus promoting engraftment, and/or might mitigate the production of HLA-antibodies (not assessed in the current study) that have been associated with graft rejection after myeloablative HLA-mismatched transplantation [51].

In conclusion, HLA-mismatched NMA HCT with MSC coinfusion appeared to be safe. Furthermore, MSC coinfusion might have prevented death from GVHD without abrogating GVT effects. Based on these encouraging results (that should obviously be confirmed in a larger number of patients), a multicenter randomized study of MSC cotransplantation in patients given HLA-mismatched PBSCs after nonmyeloablative conditioning is being developed in Belgium.

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